The Effectiveness of Immunostimulant from Ethanol Extract of Parasite Coffee Leaves in Male Rat with Slow Type Hypersensitivity Method

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Keywords: Ethanol Extract of Parasite Coffee, Volume Reduction of Swelling on a rat's feet, Slow Type Hypersensitivity.

Abstract: This research aimed to determine the effectiveness of immunostimulant from ethanol extract of parasite coffee leaves with four dose variants. This research used 18 male white rats with 200 grams of weight divided into 6 treatment groups. The rats were induced with Escherichia coli for 7 consecutive days intra-peritoneum, then on the 7th day gave a mark on the rat's feet that had been measured (V0). Ethanol extract of parasite coffee leaves with 50mg / kg weight, 100mg / kg weight, 200mg / kg weight, 400mg / kg weight, 0.5% CMC Na suspension, and 25 mg / kg weight STIMUNO suspension as positive control were given orally on 8th day, after 24 hours of administration the volume of the rat's feet was measured again, then all data from each group was processed by using the ANOVA test. The results showed that the ethanol extract of the parasite coffee leaves proved to be effective as an immunostimulant. This is attested by reduction in the volume of swelling on rat's feet which tested by the slow type hypersensitivity. It was said to be effective because it had an approaching value positive control that is 25 mg stimuno starting from 200 mgkg / weight dose to 400 mgkg / weight. With an average reduction in the volume of swelling 1.4 mm for dosage of 400 mgkg / weight and 1.2 mm for dosage of 200 mgkg / weight.

1 INTRODUCTION

The commonly treatments of the Cancer are surgery, radiotherapy and chemotherapy, but there are not getting the optimal results yet from those therapies. Each of these therapies has several side effects that tend to harm the patient. The failure that often occurs in the treatment of this cancer, mainly through chemotherapy, is caused by the low selectivity of anticancer drugs against normal cell. Moreover, the failure of chemotherapy is also caused by cancer cell resistance to chemotherapeutic agents. Treatment of this disease requires an aggressive and innovative approach to the development of new treatments that require the role of immunomodulators to enhance the immune system (Rahim, Suartha and Sudimartini, 2017). Cancer treatments that are commonly done are surgery, radiotherapy and chemotherapy, Optimal results have not been obtained from all of the three types of therapy. Every therapy has several side effects that endangers the patient. Failures that often occur in treatment the cancer, therapy through chemotherapy is due to low selectivity anticancer drugs against normal cells (Yulianti et al., 2018). This resistance phenomenon has consequences for increasing dosage of therapeutic. It can be called a Multi-Drug Resistance (MDR) phenomenon that can increase the level of toxicity of drugs that was used for therapy. Resistance to therapy is a major problem in cancer. Even if the treatment is initially successful, the tumor often remains stubborn. Chemotherapy treatments are increasingly modern even though there is no 100% effective cancer treatment for treating cancer. Drug resistance is influenced by several factors including individual factors such as somatic genetic cells in tumors.Resistance is a common thing in cancer and as cancer therapy becomes more effective, drug

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resistance is more frequent. The cause of this resistance is due to the presence of compounds in the body that detect anti-cancer drugs in the cell to expel it. Apoptosis, induction and detoxification also play a role in the formation of body resistance to drugs.The mechanism in which cancer drug resistance functions provides important information in avoiding increasing levels of resistance to chemotherapy. In addition, resistance also has implications and pharmacokinetics for drugs that are generally used (Brown et al., 2016).

Indonesia is rich in biodiversity that has the potential to be developed as a drug or drug raw material that the function as an antioxidant with low cytotoxicity. The research on plants such as herbs, spices, and plants that ride on other plants, such as parasite is widely carried out because of its potential (Wulandari et al., 2019). As an antioxidant. In Europe, parasites especially the leaves, which have been studied to be used as drugs in the treatment of cancer deadly cure. In this parasite leaf there are various kinds of substances that can help treat this dangerous disease. Since the 16th century, Europeans have used parasites as a powerful herbal remedy for treating neurological disorders such as epilepsy. For someone who has epilepsy, he tends to have difficulty controlling his own body and uses the benefits of parasitic leaves which are believed to be very effective in overcoming this health problem (Kumolosasi et al., 2018). Parasite is a semi-parasitic plant, which was originally considered a detrimental plant because it damages commercial plants. But “benalu” has the potential as a medicinal herb. Traditionally a number of parasitic species since ancient times have been used to prevent and treat various diseases including as cough medicines, cancer, diuretics, anti-inflammatory, antibacterial, wound or mold infection. The parasite is a type of plant that propagates on other plants and takes food from attached plants. Coffee parasite is a type of plant that lives attached to the coffee plant as its host. In general, this parasitic plant grows well in regions that have a tropical climate like in Indonesia. Many parasites grow on several other types of plants such as mangoes, coffee, starfruit and many others. In the past, not many people knew the benefits of this parasite coffee plant because it was considered to interfere with the plants that were used as hosts. Because this process of foraging from parasites originates from the host that is attached to it, the plant that is attached becomes lack of food reserves and cannot grow properly (Ojezele, Erhirhie and Arojojoye, 2016).

One of the plants that is very good to be a host is a coffee plant.The parasite of this coffee is usually attached to the branches and also the trunk of the coffee tree. Often this plant is found in the Mandiling Natal area in North Sumatra. The types of parasites attached to this coffee plant include parasites which have many benefits that can be used to cure various diseases. As the attached coffee plant, parasites also absorb some of the contents of the coffee plant so the parasite has more or less the same content in the coffee plant. Usually coffee plants that have the sap can eventually be used as an alternative to treating cancer through parasites. Chemical content that contained in parasites are phenolic, tannin, amino acid, carbohydrate, alkaloid and saponin. Phenolic compounds are very active as antioxidant. Phenolic compounds have a structure that can easily contribute hydrogen or electrons to acceptors such as reactive oxygen species or peroxyl groups from fat, so they can reduce the activity of oxygen and peroxyl radicals (Ojezele, Erhirhie and Arojojoye, 2016). Flavonoid is a polyphenol compound that is found in epidermis of the leaves of fruit peels and has an important role in human life as an antioxidant, antimutagenic, antineoplastic and vasodilator activity. a compound consisting of 15 carbon atoms which are generally spread over the plant world. More than 2000 plant-derived flavonoids have been identified, but there are three groups commonly studied, namely anthocyanin, flavonols, and flavones (Yulianti et al., 2018).\Chemical contents that contained in parasites are flavonoid, tannin, amino acid, carbohydrate, alkaloid, and saponin. Based on various researches, compounds in parasites that are thought to have anticancer activity are flavonoids, which are quercetin which are inhibitors of DNA enzymes for cancer cell topoisomerase. Quercetin is the main flavonoid compound contained in the parasite. The compound is a taxonomic marker of the Loranthaceae family (Hueza et al., 2019).

Parasite of coffee is a type of plant whose life does not require soil for its media. It lives as a parasite, sticks to the host cell, and absorbs the nutrients its has so that cause death to the host cell. The presence of Chlorophyll causes parasites have the ability to carry out photosynthesis. However, this plant is not able to take water and nutrients directly from the soil which makes it as a parasite plant (Poelman et al., 2019). The using of parasites as traditional medicine has been known for a long time to cure various diseases. Parasite is used by the society as an anti- inflammatory drug, pain reliever (analgesic), antivirus, anticancer, etc. For example

tea's parasite and mango's parasite which are used as the cure for cancer (Choi et al., 2019). The flavonoid content in coffee's parasite can be optimized in the extraction process by defeating (Yulianti et al., 2018). Definitely, also tumor and immune cells, including tumor associated macrophages and tumor-infiltrating lymphocytes (of both the innate and the adaptive arm of the immune system) can produce these factor (Poggi, Varesano and Zocchi, 2018).

The immune system is a body's defense system that has the function to protect, maintain and destroy antigens such as bacteria, viruses and other microorganisms that can cause various diseases. One effort to maintain the immune system is by giving (Putra, Azizah and Nopriyanti, 2020). The immune response is the response of the immune system to objects or substances that are considered foreign. So there are two types of immune responses that might occur, namely the nonspecific immune response and the specific immune response (Emelda et al., 2018). Immune system compilation exposed to substances that are considered foreign, then there are two types of immune responses that might occur, non-specific and specific immune response. Non-specific immune response is immune congenital (innate immunity) in the sense that the response to foreign substances can occur in the body has never been exposed to these substances. Meanwhile, a special immune response is acquired immune responses that arise against certain antigens (Xue et al., 2019). Immunomodulation is a compound that can affect the humoral and cellular immune systems. There are two types of immunomodulation, namely immunostimulator (boosting the immune system) and immunosupressor (suppressing the immune system). Some compounds that contained in plants have immunostimulatory and immunosuppressive effects. Immunomodulation are related to macrophage activity and capacity (Yulianti et al., 2018). Macrophage is one of the cells that play an important role in the immune response, both functional roles in phagocytosis and their role as Antigen Presenting Cells (APC). In carrying out these two roles, the endogenous mediator aid such as cytokines is definitely needed. While the need for an exogenous mediator such as carotene and flavonoid still needs thorough research (Ofokansi et al., 2018). A substance that acts as an enhancer or booster immune can be obtained by using of herbs that have efficacy as an immunostimulant. One of the herbs which used is ethanol extract of the parasite leaves of coffee which according to previous researchers has the potential as an immunomodulation with high antioxidant activity (Suparman, Kadarusman and Situmeang, 2018).

Immunomodulation is a compound that can affect the humoral and cellular immune systems. There are two types of immunomodulation, namely immunostimulator (boosting the immune system) and immunosupressor (suppressing the immune system). Some compounds that contained in plants have immunostimulatory and immunosuppressive effect, Immunomodulation is related to activity and capacity of macrophage (Rahim, Suartha and Sudimartini, 2017). Water extract of ethanol extract from parasite leaves of coffee (LoranthusferrugineusRoxb.) Active Test of immune system activity can be done by various methods, namely by looking at phagocytosis activity using carbon clearance methods, slow type hypersensitivity response, and hemagglutination test for antibody titer (Zhang et al., 2019). The effectiveness of the immune system test can be carried out with the antibody titer method determined based on the last dilution where the antibody is still detected through visually involved hemagglutination. The antibody titer value is transformed with [2log (titer) +1] (Marbun, Suwarso and Yuandani, 2018). To obtain the compound of flavonoids, the parasite leaf powder was extracted first with methanol, then the dried methanol extract was extracted again with ethyl acetate and ethyl acetate extract was partitioned with n-hexane. Residue that insoluble with n-hexane is a total flavonoide. Total flavonoids were hydrolyzed with HCl 2 N to obtain aglycone flavonoids. Methanol extract, ethyl acetate and n-hexane extract phytochemical screening to determine the class of metabolites contained therein, and carried out antioxidant activity (Eriani et al., 2018). Based on the above considerations, the authors feel it is important and necessary to test theimmunostimulator effect of ethanol extracts of the parasite leaves of coffee (LoranthusferrugineusRoxb.) in male rats. Therefore, scientific research such as immunostimulator research and testing in pharmacology is needed.

2 METHOD

The tools and materials used rotary evaporators, mercury water pletismometers, vapor cups, glass beakers, analytical weights, reaction tubes, CMC Na, 96% alcohol, Na C l, coffee leaf extract. The process of taking and preparing samples can be seen in Figure 1. The animals used 18 white rats about

150-200 grams which are treated by unifying food. Animal examples used for experiments can be seen in Figure 2a. The dosage used in the research is based on previous research on the activity and vasorelaxant of the parasite leaves of 50mgkg /weight, 100mgkg/weight, 200mgkg /weight, and 400mgkg/weight. Each group was made up of three rats. The process of giving ethanol extract of parasitic leaves of coffee (EEBK) to rat can be seen in Figure 2b.

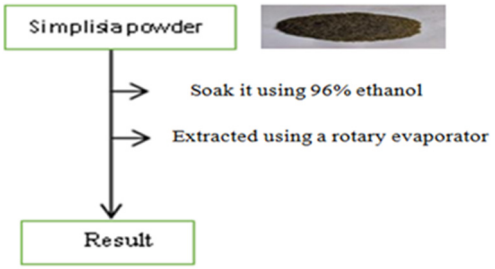

Figure 1: Taking and preparing sample

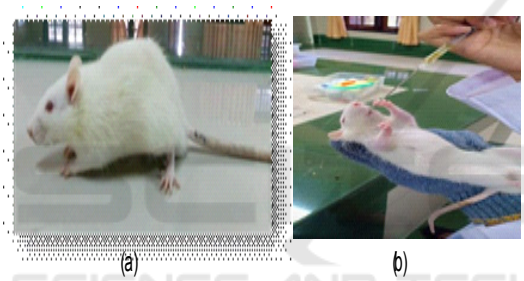

Figure 2: (a) Animal experiment; (b) Giving (EEBK) to rat

Weighed the extract as much as 50mg, was added to the mortar, then poured a little 0.5% CMC Na suspension while crushed until homogeneous, afterhomogeneous it was put into a 100ml volumetric flask. Phytochemical is used to test whether or not of secondary metabolites in plants qualitatively. The method that is used for searching and finding bioactive compounds is phytopharmacologic screening approaches. Alkaloid examination. The sample was weighed as much as 0.5 g then added 1 ml of 2N hydrochloric acid and 9 ml of distilled water, heated over a water bath for 2 minutes, cooled and filtered. The filtrate obtained was used for the alkaloid test. Then divided into 3 tubes, each tube added 2 drops of reagent mayer, 2 drops of bouchardat reagent, 2 drops of dragendroff reagent. Alkaloid is positive if there are 2 to 3 deposits in each experiment.

Flavonoid examination: The sample was weighed 5g, then added 10 ml of hot water, boil for 5 minutes and filtered under heat. Into the filtrate were powdered magnesium and 1 ml of concentrated hydrochloric acid and 2 ml of amyl alcohol. Shaken and allowed to separate. Flavonoid is positive if there is a yellow, orange or red color in the alcoholic layer. Tannin examination: The sample was weighed as much as 1g boil for 3 minutes in natural distilled water then cooled and filtered. 1-2 drops of iron (III) chloride 1% w / v are added to the filtrate. If there is a blackish blue or blackish green indicates tannin. Saponin experiment: The sample is weighed 0.5g and put into a test tube, then added 10ml of hot water, cooled, then shaken vigorously for 10 minutes. If a stable 1- 10cm high foam is formed not less than 10 minutes does not disappear with the addition of 1 drop of 2N hydrochloric acid then it shows the presence of saponin. Glycoside experiment: The sample was weighed 3g and then mixed with 30ml alcohol-water (7: 3) and 10ml of 2N hydrochloric acid, refluxed for 2 hours, cooled and filtered. Taken 20 ml of filtrate, 25 ml of distilled water and 25 ml of lead (II) acetate of 0.4 M are added, shaken, allowed to stand for 5 minutes, then filtered. The filtrate was filtered with a mixture of chloroform-isopropanol (3: 2) 3 times. Anhydrate sodium sulfate is added to the extract, filtered and evaporated at a temperature of no more than 50ºC. The remainder is dissolved with 2 ml of methanol, the remaining solution is put into a test tube, evaporated on a water bath. To the remaining, added 2 ml of water and 5 drops of molish reagent. Be safe to added 2 ml of concentrated sulfuric acid, a purple ring formed at the liquid limit indicates the presence of a sugar bond. Steroid examination: The sample was weighed as much as 1g, macerated with 20 ml of n-hexane for 2 hours, filtered. The filtrate was evaporated in a vaporizer cup and the remaining concentrated sulfuric acid reagent was added through the wall of the cup. If a purple or red color turns into purple or blue, green, it indicates the presence of steroids / tri terpenoids.

The phytochemical test results of the parasite leaves sample contained alkaloids, tannins, saponins, glycosides and flavonoid. Phytochemical test results on leaves are slightly different from those ever reported by muamar muulian who stated that secondary metabolites contained in methanol extracts of the parasite leaves of coffee are alkaloids, flavonoids, terpenoids, and tannins. This is expected because the samples obtained are from different places so that the content of secondary metabolite is different.(Yulianti et al., 2018). Phytochemical screening test results can be seen in Table 2 and recording the results of the swollen volume of rat feet can be seen in Table 1.

Table 1: Recording of the results of the volume of swelling of the rats feet.

No	Doses	Rat	Swelling volume of rat feet			Average
			Before	After	Deviation	
1	50 mg/kg weight	1	2,12	1,2	0,92	0,92
		2	2,12	1,2	0,92	
		3	2,13	1,2	0,93	
2	100 mg/kg weight	1	2,12	1,2	0,92	0.95
		2	2,12	1,14	0,98	
		3	2,13	1,19	0,94	
3	200 mg/kg weight	1	2,12	0,92	1,2	1,2
		2	2,12	1,02	1,1	
		3	2,13	0,93	1,2	
4	400 mg/kg weight	1	2,12	0,82	1,3	1,4
		2	2,12	0,72	1,4	
		3	2,13	0,83	1,3	
5	STIMUNO	1	2,12	0,45	1,67	1,6
		2	2,12	0,47	1,65	
		3	2,13	0,48	1,65	
6	BLANKO	1	2,12	1,47	1,65	0,64
		2	2,12	1,4	0,63	
		3	2,13	1,48	0,65	

Table 2: Phytochemical screening test

No	Skrining	Ekstrak
1	Alkaloid	Positif
2	Flavonoid	Positif
3	Tanin	Positif
4	Saponin	Positif
5	Glikosida	Positif
6	Steroid/triterpenoid	Negatif

Test animals used 18 male rats with a weight of 150-200 grams. Before treatment, the experimental animals were first conditioned for 2 weeks in a good cage to adjust their environment and uniform food. The animals were grouped into 6 groups including 1 group with 0.5% CMC Na absorption, the second group with a 25mg stimuno suspension, the third with 50mgkg /weight ethanol extract suspensions, the fourth with a leaf ethanol extract suspension parasite's coffee with a dose of 100mgkg/weight, the fifth with ethanol extract suspension of the parasite leaves of coffee at a dose of 200mg/weight, the sixth with the suspension of ethanol extract of the parasite leaves with a dose of 400mg/weight. Each group of experimental animals were injected e.coli inoculums intravenously as antigens with 0.1 inoculum, treatment was started on day 0. The process of the slow type hypersensitivity testing method can be seen in Figure 3.

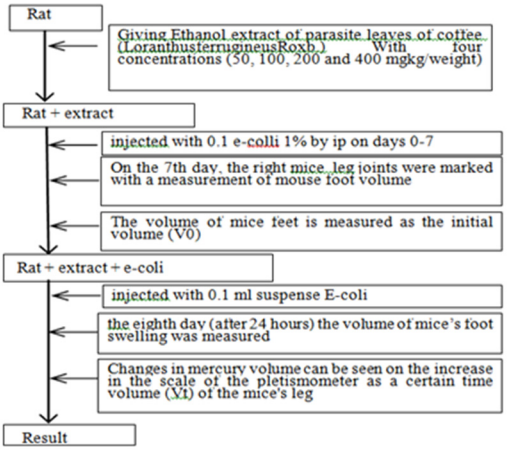

Figure 3: Slow Type Hypersensitivity Testing Method.

Homogeneity and normality are determined by the research results to determine the statistical analysis used. If there are differences, proceed by using the Post Hoc Tuckey test to find out which variables have differences. Data were analyse using the one-way ANOVA test to determine the average difference between treatments. Based on the significance value of $p \leq 0.05$, it is considered significant. The type of research used is Laboratory Experiment. This research uses Anova One Way parametric test. The sample which used in this study was the Ethanol Extract of Parasite coffee Leaves (LoranthusferrugineusRoxb). The data obtained were analyzed using SPSS with the Anova test. Continued by the Post Hoc Tukey test to see variables that have a significance value of $p < 0.05$.

3 RESULT AND DISCUSSION

Phytochemical test results of the parasite leaves of coffee containing alkaloids, tannins, saponins, glycosides and flavonoids. Phytochemical test results on leaves are slightly different from those ever reported by Yullian Muamar who stated that secondary metabolites contained in methanol extracts of the parasite leaves of coffee are alkaloids, flavonoids, terpenoids and tannins. This is expected because the samples that obtained are from different places so that the content of secondary metabolite is different.(Yulian, 2018). From 1500 grams of dried samples macerated using a rotary evaporator to get 30 grams of ethanol extract of parasitic coffee. Thick extract has a distinctive odor, black in color.The result of this research of ethanol extract of parasite coffee leaves is given for 7 days at a dose of 50 mg /

BW and a dose of 100 mg / BW still provide effects such as negative control. However, at a dose of 100 mg / BW has begun to lead to positive control, at a dose of 400 mg / BW the measurement results of the swelling feet volume of rat approach the positive control of stimuno with an average value of 1.6, with an average of 1.4. We strongly encourage authors to use this document for the preparation of the camera-ready. Please follow the instructions closely in order to make the volume look as uniform as possible (Breda et al., 2019). Based on the results of the research that has been done by varying the dosage of ethanol extract of parasites of coffee leaves by measuring the volume of rat swelling using a mercury pletismometer, using the slow type hypersensitivity method is found in Figure 4.

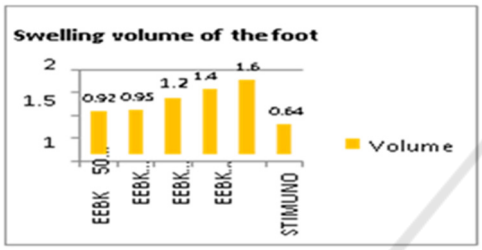

Figure 4: Swelling volume of rat feet.

The results of macroscopic examination carried out on the parasite leaves of coffee (LoranthusferrugineusRoxb.) Namely the top surface is smooth and green, the lower surface is weak green. The macroscopic results of the form of coffee parasites are slim-shaped roots, radiating at the host and dull in color. Long stems of plants upright dull green small, oval shaped leaves that have a dark green color with a slightly rough surface. There are small seeds - interrupted between leaf stalks and stems, small seeds shaped like the contents of a pencil, have a short tentacle, macroscopic and microscopic examination carried out on parasites of coffee leaves in Figure 5 and Figure 6.

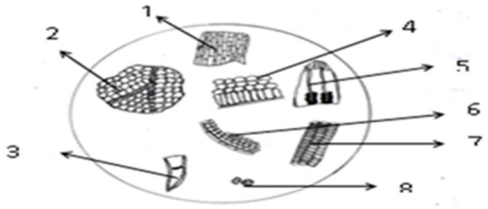

Figure 5: Macroscopic examination carried out on parasites of coffee leaves.

Description Figure 5: Leaf stalk parenchyma (1), Leaf parenchyma with calcium oxalate crystal (2), Hair cover (3), Palisade fragments and leaf parenchyma (4), Flower petals fragments with petals (5), Pericarp parenchymal fragments (6), Trachea (7), Pollen (8).

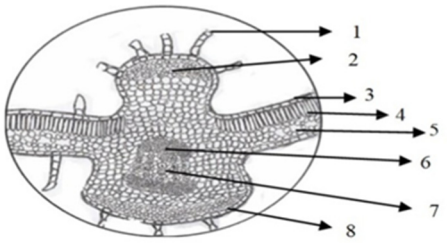

Figure 6: Microscopic examination of the parasites of coffee.

Description Figure 6: Hair cover (1), Kolenkim (2), Upper epidermis (3), Palisade parenchyma (4), Spongy tissue (5), Floem (6), Xilem (7), Lower epidermis (8).

The results of the research were obtained from the administration of ethanol extract of the parasite leaves of coffee (LoranthusferrugineusRoxb) at a dose of 50 mgkg /weight, 100 mgkg / weight, 200 mgkg /weight, and 400 mg/weight for 7 days showed a decrease in swelling volume, this can be seen in the Table 3. However, the results of several dose variants showed an average difference between each treatment.Comparison of the average value of eachtreatment that is at a dose of 50 mg / BW and 100 mg / BW that tends to have an average value approaching negative control, namely CMC Na, but at a dose of200 mg / BW to a dose of 400 mg / BW the average value changes towards the direction of positive control. There is a difference in the average value of each test group that has been done. Then it will be continued by using the post hock tukey test to find out which variable has a difference based on the significance value<0.005 which is considered significant, this can be seen in the Table 4.

Table 3: Changes in swelling feet volume of rat.

Changes in swelling feet volume of rat Tukey HSD					
Doses EEBK	Subset for alpha =0,05				
	1	2	3	4	5
Blanko3	,6433				
EEBK 50/kgBW3		,9233			
EEBK 100/kgBW3		,9467			
EEBK 200/kgBW3			1,166		
EEBK 400/kgBW3				1,333	
Stimuno3					1,656
Sig.	1,000	,964	1,000	1,000	1,000
Means for groups in homogeneous subsets are displayed					
Uses Harmonic Mean Sample Size = 3,000					

Table 4: Results of data analysis.

ANOVA					
Changes in the volume of swelling of rat feet					
	Sum of squares	df	Mean squares	F	Sig
Between Groups	1,894	6	,379	287,648	,000
Within Groups	,016	12	,001		
Total	1,909	18			

From the results of the post hock tukey test between the negative control group, the 50 mgkg / weight dose and the 100 mgkg /weight dose had an effect but was not significant compared to the 200 mgkg /weight dose and the 400 mg kg / weight dose which gave an average value close to the 25 mg Stimuno positive control. Decrease in the volume of swelling of rat's feet is suspected because in the extract contained secondary metabolites such as alkaloids, flavonoids, tannins, saponins, and glycosides contained in coffee parasites leaves (LoranthusferrugineusRoxb). This compound belongs to the group of polyphenols which besides having biological functions such as improving glucose metabolism as well as antioxidants and immunostimulants.

4 CONCLUSIONS

The results showed that the using of ethanol extract of parasite leaves at a dose of 400 mgkg/weight could reduce the swelling volume of rat's feet and was effective as a significant immunostimulant can be seen from the significance value of $p < 0.005$. The recommended dose used as immunostimulant is the highest dose. At the highest dose in this research, it has the same effectiveness with positive controls that already exist in the market, namely immunstimulants.

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